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United States Department of Agriculture,

BUREAU OF CHEMISTRY.

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I. RECENT METHODS SUGGESTED FOR THE DETECTION OF FORMALDEHYDE AND SACCHARIN.

During the last few years a number of controversies have arisen with regard to the presence or absence of preservatives in certain articles of food, and have given increased interest to the study of the methods for the detection of such preservatives. In order to promote this work as far as possible a number of the methods that have been suggested during the last few years have been abstracted and are given in the following pages.

Those receiving this circular are requested to try as many of the methods as possible and report their results to the referee at as early a date as practicable, not later than September 1, 1904. It is suggested that those whose time will not permit them to study all of the methods outlined below confine their attention exclusively to one of the two preservatives given, and that the methods given in Bulletin 65 of the Bureau of Chemistry be also examined in comparison with those given below. Quantitative methods for the determination of the small amounts of the ordinary food preservatives usually employed with foods are also greatly to be desired, but in the opinion of the referee qualitative methods are more important at the present time. It is suggested that all the formaldehyde reagents be also examined with reference to the reactions with ordinary aldehydes other than formaldehyde, and that all reagents for saccharin be examined with reference to their reactions with benzoic acid.

The question of the detection of preservatives is now one of the most important ones in food chemistry as applied to the detection of adulteration, and it is hoped that all who find it possible will take part in this work.

W. D. BIGELOW,
Referee on Food Adulteration.

JUNE 15, 1904.

FORMALDEHYDE.

E. RIEGLER.

Pharm. Centr., 1900, **40**: 769.

Formaldehyde and other aldehydes yield a rose-red color when treated with phenylhydrazin hydrochlorid and sodium hydroxid. Two cubic centimeters of milk, 2 cc of water, and 1 gram of phenylhydrazin hydrochlorid are placed in a test tube and shaken until the latter is dissolved, then 10 cc of a 10 per cent sodium hydroxid solution are added and the mixture shaken for $\frac{1}{2}$ minute. Milk containing 2 drops of commercial formalin in 100 cc takes on a rose-red color in from 2 to 10 minutes.

MANGET AND MARION.

Compt. rend., 1902, **135**: 584; abs. Chem. Centrbl., 1902, pt. 2, p. 1276.

To detect formaldehyde in milk the author treats the milk with a small amount of amidol (diamadiophenol—1, 2, 4) or amidophenol. After a few minutes, milk containing formaldehyde takes on a canary-yellow color. Even in dilution of 1 part in 50,000 the reaction is distinct. For detecting formaldehyde in meat jellies a few crystals of amidol are added to bouillon and shaken; in the presence of formaldehyde a yellow color is formed which becomes a dark yellow on the addition of ammonia. In the absence of formaldehyde the color first formed is bright reddish-brown which is converted into blue by the addition of ammonia.

A. GUSTAVE LUEBERT.

J. Amer. Chem. Soc. 1901, **23**: 682.

Five grams of milk are placed in a flask with 5 grams of coarsely powdered potassium sulphate; 10 cc of sulphuric acid are poured down the side of the flask. If formaldehyde be present the potassium sulphate becomes violet in a few minutes, and the color gradually disperses through the entire liquid. If absent a brown color forms which rapidly changes to black.

EDUARD BAIER.

Zeit. Fleisch. Milchhyg, **11**: 70; abs. Chem. Centrbl., 1901, pt. 1, p. 595.

The sample is finely macerated with an equal amount of water, the mixture filtered and the filtrate treated with a saturated solution of bromin in potassium bromid until all proteids and coloring matter are precipitated and the liquid contains an excess of bromin as indicated by the color. The mixture is then filtered and 2 cc of the filtrate mixed with 2 drops of an aqueous 1 per cent solution of resorcin and the product so obtained treated with an equal volume of concentrated sulphuric acid, care being taken not to mix the two layers. At the point of union of the two liquids a white cloud appears which is heavy or light according to the relative amount of formaldehyde present. In the middle of the white zone is a carmin red color and below is a range of yellow liquid. In great dilution the turbidity is scarcely noticeable and the reaction takes place much more slowly. The reaction takes place in the presence of formaldehyde with spoiled meat, meat preserved with sulphites, smoked meat, fresh meat, and with milk. In the examination of milk the greater part of the casein is first coagulated by means of rennet, the filtrate from which is handled as above. Even in the absence of formaldehyde a brown color is formed which obscures the carmin red color but not the white cloud. One part of formaldehyde may be detected in 25,000 parts of the solution.

C. ARNOLD AND C. MENTZEL.

Zeit. Nahr. Genussm., 1902, **5**: 353.

A red color is given by formaldehyde with ferric salts, especially in alcoholic solutions. Mix 5 cc of an alcoholic solution of the substance with 0.03 gram of phenylhydrazin hydrochlorid and 4 drops of ferric chlorid solution; from 10 to 12 cc of concentrated sulphuric acid are then added, keeping the mixture cool. An intense red color is formed. A precipitate is formed which can be dissolved by the addition of either concentrated sulphuric acid, keeping the liquid cool, or by alcohol. With meats and fats the formaldehyde should first be extracted with alcohol and the filtrate tested. Milk is shaken with an equal volume of absolute alcohol and the filtrate employed. In pale beers the coloring matter may be seen without previous treatment, and in dark beers in the froth when the mixture is shaken. In very dark beers it is necessary to add the reagent as directed above and extract with ether, subsequently evaporating the ethereal extract to dryness and treating the residue with alcohol and concentrated sulphuric acid, when the color reappears.

C. ARNOLD AND C. MENTZEL.

Chem. Zeit., 1902, **26**: 246.

Abs. J. Chem. Soc., 1902 (2), **82**: 367; abs. Chem. Centrbl., 1902, pt. 1, p. 1077.

The following tests may be applied directly to liquid foods or an aqueous or alcoholic extract of solid foods:

In 3 to 5 cc of the liquid (milk, for instance) is dissolved a lump of phenylhydrazin hydrochlorid the size of a pea, 2 to 4 drops (not more) of a 5 to 10 per cent solution of sodium nitroprussid are added, and finally 8 to 12 drops of 10 to 15 per cent sodium hydroxid. In the presence of even traces of formaldehyde, a fairly permanent blue or bluish-green color is produced. The test is rendered still more delicate by substituting potassium ferricyanid for the nitroprussid, but it can not then be recommended for milk or meat preparations containing blood-coloring matter; in case of an alcoholic solution, this must be well diluted with water to prevent precipitation of potassium ferricyanid.

Milk is examined directly. Meat is finely comminuted, extracted with 2 volumes of hot water, and the liquid pressed out and employed for the test. Fats (5 to 10 grams) are warmed above the melting point with 10 cc of from 80 to 95 per cent alcohol, thoroughly shaken, cooled, poured through a moistened filter, and the filtrate employed.

E. RIMINI.

Ann. di Farmacol., 1898, 97; abs. Chem. Centrbl., 1898, pt. 1, p. 1152; abs. J. Soc. Chem. Ind., 1898, 17: 697.

The author gives two extremely sensitive color reactions, which are characteristic for formaldehyde only, and are specially suitable for the detection of the latter in milk. (1) Fifteen cubic centimeters of a very dilute formaldehyde solution are treated with 1 cc of a dilute solution of phenylhydrazin hydrochlorid, then with a few drops of freshly prepared sodium nitroprussid solution, and finally with concentrated caustic soda solution. A blue color is formed, which, after a long time, changes to red. This reaction is capable of indicating formaldehyde in milk, even in a dilution of 1 in 30,000. (2) The second reaction is obtained in a similar manner, except that iron chlorid is used instead of sodium nitroprussid, followed by concentrated hydrochloric acid, instead of caustic soda. This gives a red color, which changes, after some time, to orange yellow.

D. VITALI.

Boll. Chem. Farmac., 1898, 37: 321; abs. J. Soc. Chem. Ind., 1898, 17: 955.

If to a weak solution of formaldehyde an equal bulk of a 4 per cent solution of a phenylhydrazin salt be added, a white cloudiness occurs, gradually becoming yellow, and after some hours yellowish-red. The turbidity occurs at once in a 1 per cent solution of formaldehyde, while in a solution containing 1 part in 100,000 it appears after two or three hours. The amorphous solid which settles out is soluble in alcohol, and can be obtained in rhombic tables by evaporating this solution. If the mixture of formaldehyde and phenylhydrazin solution be allowed to evaporate to dryness, and the washed residue taken up with alcohol, the yellow solution reduces a drop of gold chlorid and gives a greenish liquid, which is reddened by sodium hydroxid solution. If the mixture of formaldehyde and phenylhydrazin solution be mixed, immediately on the appearance of the turbidity, with sodium nitroprussid and sodium hydroxid, a splendid blue color is struck. This reaction can be used to detect formaldehyde in the presence of acetaldehyde, if the latter be not present in too great quantity, as acetaldehyde gives no color under the circumstances.

II.—METHODS FOR THE DETECTION OF SACCHARIN.

It has been recognized for some time that a substance occurred naturally in many wines which had all of the reactions of saccharin. This substance has been called false saccharin. It occurs in such slight quantity that there is no possibility of its leading to erroneous results by giving reactions for saccharin in the ordinary methods with wines to which saccharin has been added.

Blarez¹ and Carlos^{2 3} suggested that wines to be tested for saccharin be oxidized with potassium permanganate. F. de Bounge⁴ directs that in testing wines for saccharin they be first acidified with sulphuric acid and treated with a 1 per cent solution of potassium permanganate to slight excess for the destruction of salicylic acid and tannin. These methods have been carefully studied by Mr. E. M. Chace of the Food Laboratory, Bureau of Chemistry, who suggests the following details of manipulation:

Fifty cubic centimeters of wine are acidified and extracted with ether. The extracted matter is tested in the usual way for salicylic acid, the gasoline extract being returned to the dish containing the residue, the whole diluted to about 10 cc volume, and 2 cc one-third sulphuric acid added. The solution is brought to the boiling point, and concentrated potassium permanganate added drop by drop to slight excess, the solution partly cooled, a piece of sodium hydroxid added and filtered into a silver dish (crucible lids are used in this laboratory), evaporated to dryness and heated for twenty minutes at 210 to 215° C. The residues are dissolved in water, acidified and extracted with ether, the ether evaporated and residues tested with two drops of a 0.5 per cent solution of ferric alum.

¹ Rep. de pharm. (3) 6: 111.

² La revue commerciale et coloniale, December 12, 1903.

³ Rep. de pharm. (3) 6: 110.

⁴ Bull. soc. chim. 1903, (3) 29: 411.

By this method, all the so-called false saccharin, and the salicylic acid up to 10 milligrams per liter are destroyed, while one-half milligram of saccharin per liter is detected with absolute certainty, and one-eighth milligram per liter is detected in the majority of tests.

A. LEYS.

Ann. de chim. anal., 1901, **6**: 201; abs. Analyst, 1901, **26**: 321.

If 10 cc of a very dilute solution of copper sulphate or of a ferric salt be mixed with two or three drops of hydrogen peroxid, and warmed with an aqueous solution of an unsaturated organic compound, an intense brown coloration is produced. This reaction is obtained with salicylic acid, benzoic acid, cinnamic acid, saccharin, acetanilid, ortho-phenylene-diamine, antipyrine, pyrrhol, thiophen, and furfural. No color is obtained with alcohol, acetic acid, lactic acid, sucrose, allyl acetate, fumaric acid, menthol, or turpentine.

The brown coloration given by saccharin can be prevented by the addition of a trace of sulphuric acid, and even after warming the mixture for a few moments only a faint yellow tint is produced. After a short time two drops of a very dilute solution of ferric chlorid are added, and a violet coloration is obtained. This reaction, which is due to formation of oxybenzoic acid, is also given by benzoic acid. By using very dilute solutions of ferric chlorid and hydrogen peroxid in the cold, the addition of the acid is rendered unnecessary.

Saccharin in milk.—Fifty cc are treated with 100 cc of a 10 per cent solution of potassium bisulphate to each 100 cc of which has been added 10 cc of 99 per cent alcohol, and the precipitated casein filtered off. The filtrate is shaken with two portions of ether, crystals of bisulphate that may separate during the second extraction are redissolved with a few drops of alcohol, the ethereal extracts are evaporated to dryness at 90° C, and the residue dissolved in 5 cc of boiling water. When cold the solution is tasted, and, if sweet, is tested with two drops of a very dilute solution of ferric chlorid, and 2 cc of dilute hydrogen peroxid, and left for thirty minutes. In the presence of saccharin a violet tint is slowly developed.

Saccharin in butter.—Dissolve the butter in a mixture of equal volumes of chloroform and absolute alcohol; add twice the volume of water, shake, allow to settle, and separate. The separated chloroform retains the fats; the aqueous-alcoholic solution contains the saccharin. Evaporate the latter, and test as above.

The ferric chlorid solution is prepared by diluting 2 cc of a solution of 30° Bé. to 100 cc, and the hydrogen peroxid by diluting 1 cc of the ordinary 10 volume (3 per cent) solution to 200 cc.

MATTEO SPICA.

Gazzetta, 1901, **31**, ii, 41; abs. J. Chem. Soc. 1901, pt. 2, **80**: 704.

The author has devised two methods for the detection of small proportions of saccharin in commercial products. The first of these consists in converting the imino group of the saccharin into nitric acid, which is recognized by means of diphenylamine hydrochlorid, whilst in the second method the saccharin is transformed into one of the sulphaminobenzoic acids, which can be detected by the diazo-reaction. The method of working is as follows: The liquid to be tested, acidified with sulphuric acid, or in the case of a solid, the extract obtained with the dilute acid, is shaken in a separating funnel with ether or a mixture of ether and light petroleum, the liquid being then filtered and divided amongst three dry test-tubes, which are placed in a water-bath to evaporate. The residue in one tube is tested for salicylic acid by adding nitric acid and heating gently to form picric acid, which is recognized by the method previously described.¹ To the second residue is added a little lime, best somewhat granular as obtained from powdered marble, and the mixture then heated until it becomes slightly brown; a few cubic centimeters of water are then added, the liquid heated to boiling, and the clear solution decanted off into another tube, where it is mixed with a few drops of hydrochloric acid and a small piece of zinc; after hydrogen has been evolved for twenty minutes, the liquid is again decanted off and to it are added a few drops of dilute sodium or potassium nitrite solution and 5 to 6 drops of a naphthylamine hydrochlorid solution; in the event of "saccharin" being present in the substance examined, a crimson coloration is obtained which only appears after some hours if the quantity of "saccharin" is very small. To the residue in the third tube are added a few drops of pure sulphuric acid and a crystal of potassium permanganate, oxidation being started by gentle heating,

¹ Abs. J. Chem Soc., 1895, pt. 2, p. 426.

and the excess of permanganate removed by means of oxalic acid or sulphur dioxid. The liquid is then diluted with a few cubic centimeters of water, a few drops of diphenylamine hydrochlorid are added, and pure sulphuric acid poured carefully down the side of the test-tube so as to form a distinct layer at the bottom. The presence of nitric acid, produced from "saccharin" is manifested by an azure-blue ring in the layer between the sulphuric acid and the aqueous liquid.

E. RIEGLER.

Pharm. Centralh., 1900, **41**: 563; abs. Chem. Centrbl., 1900, pt. 2, p. 880.

If from 0.01 to 0.02 gram of saccharin be dissolved in 10 cc of water and be treated with 2 drops of 10 per cent sodium hydroxid solution, and *p*-diazonitroanilin be added, drop by drop, with constant shaking, a greenish yellow liquid will first form and then disappear. The liquid is then shaken with 10 cc of ether, and from 20 to 30 drops of 10 per cent solution of sodium hydroxid added. At point of contact between the ether and sodium a fine green ring is formed and on shaking the ether turns green and the alkali a yellowish brown. If the aqueous layer be removed and 5 cc of concentrated ammonia are added to the ethereal portion, the latter is decolorized while the ammoniacal portion turns a distinct green.

Salicylic acid treated in the same manner gives an intense red ring and a red aqueous solution, but the supernatant ether is colorless. On treatment of ethereal layer with ammonia the ether remains colorless, but the ammoniacal liquid turns red. If saccharin and salicylic acid are both present a red, though somewhat brown, ring is first obtained, the ether is turned green and the aqueous solution red. On the addition of ammonia the ether becomes colorless and the aqueous liquor violet. The color of the aqueous layer varies in shade according to the relative amounts of salicylic acid in saccharin present. The reagent is prepared by dissolving 2.5 grams of *p*-nitranilin in 25 cc of water and 5 cc of strong sulphuric acid. When the solution becomes clear, 25 cc of water are added and 1.5 grams of sodium nitrite dissolved in 20 cc of water are also added and the whole diluted to 250 cc. This solution may be kept for a long time in the dark without being decomposed. If it becomes turbid it may be filtered and the filtrate may still be employed.

OFFICIAL RUSSIAN METHOD.

Zeit. Ver. deut. Zucker-Ind., 1901, **38**: 95.

The following is the official Russian method as adopted by the customs authorities for the detection of saccharin in food:

Solid and sirupy substances are first diluted with two parts of water, filtered, and the filtrate tested. In the case of beer and other substances containing hops a saturated solution of copper nitrate is added until no further precipitate is formed. In all cases where tannin is present a small amount of ferric chlorid solution is added and the liquid made slightly alkaline with calcium carbonate and filtered for the purpose of removing tannin. The following methods are recommended for the detection of saccharin:

1. One hundred cubic centimeters of the liquid are acidified with hydrochloric acid, treated with an excess of bromin water, well shaken and filtered. The filtrate (or supernatant liquid removed by decantation) is treated with a current of air to remove the bromin, extracted with ether, and the presence of saccharin detected in the ether extracted by the taste and by the presence of salicylic acid after fusing with caustic potash.

2. One hundred cubic centimeters of the liquid are made strongly acid with hydrochloric acid and shaken with equal parts of ether and petroleum ether. The residue from the volatile solvent is converted into salicylic acid by fusing for one-half hour in a glycerin bath at 250° with caustic soda.

Salicylic acid, if present, is removed as the bromin compound by the first method.

Tests for saccharin.—The ether extract is evaporated and the residue is warmed with resorcinol and concentrated sulphuric acid and the mixture poured into an excess of caustic soda solution. The presence of saccharin is indicated by a strong fluorescence which is interfered with by succinic acid when present.

Second method.—The substance is treated with concentrated nitric acid, the solution evaporated to dryness and a few drops of a solution of caustic potash in 50 per cent alcohol added. On rewarming, a blue color changing to violet, then to purple red, and finally to red, is formed in the presence of saccharin.

H. DEFOURNEL.

J. pharm. chim., 1901, **13**: 512; abs. Chem. Centrbl., 1901, pt. 2, p. 60.

The liquid is acidified with sulphuric acid and extracted with ether as usual or with a mixture of equal parts of ether and petroleum ether. The volatile solvent is separated, washed with water to remove acids, and evaporated to dryness. The residue is treated with ammonia, the excess of which is expelled by evaporation on the water bath. It is then dissolved in a small amount of water and transferred to an ordinary urometer, in which it is decomposed by means of hypobromite. The volume of nitrogen expressed as tenths of a cubic centimeter, divided by 8.9, gives the weight of saccharin in centigrams.

III. THE DETECTION OF SALICYLIC ACID, BENZOIC ACID AND SACCHARIN IN THE PRESENCE OF EACH OTHER.

This subject is one which requires much study and to which the attention that has been given has been entirely insufficient. The following abstracts are given with the hope that they, together with the methods given on pages 3 and 5, may lead others to undertake a careful study of this subject:

SACCHARIN: ESTIMATION OF, IN THE PRESENCE OF SALICYLIC ACID.

This method depends on the insolubility of the bromin compound of salicylic acid in water. One hundred cubic centimeters of the liquid under examination are acidified with hydrochloric acid and an excess of bromin water added, the whole well shaken and after a short time filtered. The filtrate is then treated with a current of air to drive out the excess of bromin and extracted with ether. The ethereal solution is then used for the detection or estimation of saccharin by any of the ordinary methods.

SEPARATION OF SALICYLIC ACID AND BENZOIC ACID.

J. SCHAAP.

Ned. Tydschr. Pharm., 1902, through Chem. News, **66**: 42.

The author suggests the separation of salicylic and benzoic acid by means of the insolubility of the salicylic acid compound with bromin. The solution is treated with an excess of bromin water and the precipitate removed by filtration. This is washed, dried in a desiccator and weighed. The filtrate is made slightly alkaline with sodium carbonate and evaporated almost to dryness. It is then acidified with hydrochloric acid, and benzoic acid extracted with chloroform. The chloroform may then be volatilized and the amount of benzoic acid determined by weighing.

